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## THE DEVELOPMENT AND PRESENT STATUS OF THE GENE CONCEPT

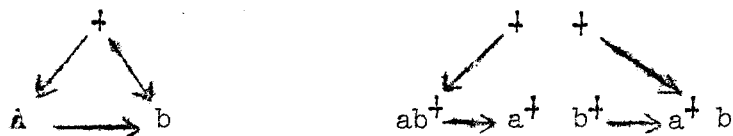
Abstracted by Claude Hinton and Joseph Mandell

One of the outstanding problems of biology today concerns the nature of the gene - its structure, its replication, its function. The first half-century of genetic research achieved an understanding of the mechanisms of inheritance, and the development of the gene concept was intimately related to this achievement. This concept defined the gene as a unit of crossing over, as a unit of mutation, and as a unit of function. However, recent studies of gene action have made it necessary to reexamine these three related criteria by which we recognize a gene.

The gene as a unit of crossing over. In his classic studies on edible peas, Mendel was unhampered by linkage between his factors; they were all in different chromosomes and therefore segregated independently. Soon after the rediscovery of Mendel's work, it was realized that there must be more genes than chromosomes in an organism. Linkage in the sweet pea was reported by Bateson and Punnett in 1906, and in 1910 Morgan studied recombination between white and rudimentary, two sex-linked mutants in Drosophila melanogaster. Morgan deduced that the amount of recombination between two genes must be a function of their distance apart, and Sturtevant conceived of the linear arrangement of the genes in the chromosomes. These studies led logically to the characterization of the gene as a unit of genetic exchange, within which no crossing over occurs. Characters which exhibit recombination between them, i.e., crossing over, are considered to be determined by two or more single units.

Conversely, characters which do not exhibit crossing over are considered to result from the action of a single unit. Clearly, defining this unit is a function of the extent of the search for crossing over, and so the unit may be subject to subdivision.

The gene as a unit of mutation. Early in the course of *Drosophila* studies, several mutants were found which affected eye color in different degrees and which showed no crossing over between them. This finding of multiple alleles implied that a single gene might occupy one of several alternative functional states. Consideration of the process of change from one state to another, i.e., mutation, resulted in the conclusion that the gene is a unit of mutation. For example, two mutants,



a and b, both originated independently from wild type and behave as alleles. Further, a is known to mutate directly to condition b. This last fact argues for a single element capable to mutating in different directions. If two elements were involved, then two mutational steps would have been required for the change from a to b.

The gene as a unit of function. The study of multiple alleles also led to this third criterion in the gene concept. Considering only recessive alleles, we find that they are more or less alike in their phenotypic expression, which suggests a similarity in functional behavior. The genetic test employed to establish allelism consists of obtaining the heterozygote for two suspected mutants, say  $a_1$  and  $a_2$ . If these are alleles, they must occupy the same locus in homologous chromosomes, and no crossing over between them will be detected.

The alternative heterozygotes may be diagrammatically represented as  $\frac{a_1}{a_2}$  for the case of alleles and as  $\frac{a_1 +}{+ a_2}$  for nonallelic mutants. More important from the standpoint of gene function, however, is the phenotype of the heterozygote. In the case of allelism, the phenotype is typically intermediate between the phenotypes of either allele when homozygous; if  $a_1$  and  $a_2$  are non-allelic, the heterozygote will exhibit a wild-type phenotype. Thus the functional identity suggested on the basis of phenotypic similarity of multiple alleles is supported by their interaction in the heterozygote to produce also a similar phenotype. As a corollary, we expect no interaction between non-allelic recessives in the heterozygous condition since they are concerned with different functions, each of which is controlled by a dominant wild-type allele.

From these and other lines of study a unified theory of the gene was developed. While subsequent observations have generally supported and strengthened these concepts of the gene, some flaws have appeared, especially in relation to the criterion of the gene as a unit of function.

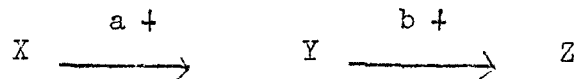
Position Effects. The discovery (1925) of the position-effect phenomenon presented the first complication in the view of the gene as a functional unit. It was found that the phenotypic expression of two doses of the mutant Bar depended upon their relative positions. If the mutants were both in the same homolog (as a result of unequal crossing over) the phenotype was less extreme than if each homolog carried one dose. Thus the expression of a mutant depends not only on its constitution, but also upon its position.

Many examples of position effects have since been studied in *Drosophila*, the most numerous type being those associated with gross rearrangements of the chromosomes. As an example of this type, consider the hypothetical case of a translocation (T) which removes the dominant wild type gene A from its normal position to another chromosome. In the heterozygote T(A)/a, A no longer acts as a dominant, but rather the heterozygote exhibits the mutant (a) phenotype as though A had mutated to a. That A has not mutated can be shown by removing it from the translocation to its normal position, where it once more functions as a dominant. Here again, the expression of a gene has been altered by its position.

Position Pseudoallelism. In recent years several cases have been investigated in *Drosophila*, *Aspergillus* and *Neurospora* which have led to further elaboration of the functional behavior of the gene. These cases consist of mutants which behave as alleles according to their similar phenotypic expression, map position, and the classical test for allelism; however, these "alleles" are separable by crossing over with a very low frequency (ca. 1/10000). Thus the two criteria of the gene as a unit of function and crossing over are in disagreement. A second exceptional feature of these pseudoalleles is the finding that their function depends upon their positions with reference to each other. Whereas the phenotype of the heterozygote  $\frac{a \text{ } +}{+ \text{ } b}$  is mutant, the alternative heterozygote  $\frac{a \text{ } b}{+ \text{ } +}$  is wild type.

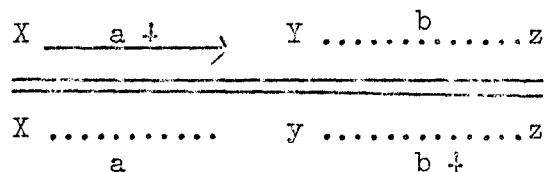
A biochemical model has been proposed to account for the functional behavior of pseudoalleles. A two-step sequential reaction is postulated in which each step is controlled by one member of the pseudoallelic pair,

and the product Y of the first reaction is the substrate

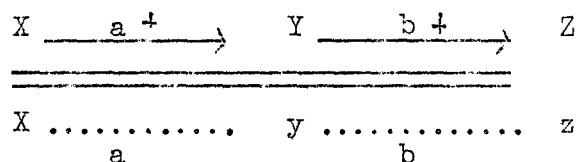


of the second. It is further required that this reaction proceed in the immediate vicinity of the controlling alleles and that product Y be either highly labile, non-diffusible or produced in very limited quantity.

Thus in the trans configuration of the alleles product Y is



not available as a substrate in the homologous chromosome, and as a consequence no product Z and a mutant phenotype result. In the cis



configuration, both steps of the reaction proceed in the same homolog to produce product Z and a normal phenotype. It is conceivable that this model might represent either successive enzymatic reactions, or successive steps in the formation of an enzyme. The model may be extended to a three-step reaction for the case of three pseudoalleles.

From the point of understanding the relationship of the immediate gene products of pseudoalleles, microorganisms are potentially useful because of the relative facility with which biochemical investigations can be made. The large population obtainable from microorganisms presents another advantage in the detection of pseudoallelism inasmuch as crossing over between pseudoalleles occurs at a very low frequency.

In all cases of pseudoallelism studied in *Drosophila*, it has been possible to relate the pseudoallelic loci to cytologically visible bands

in the salivary gland chromosomes. At present, we can only say that there may be a one-to-one relationship between visible bands in the chromosomes and genes.

Conclusion. Studies on position effects and position pseudoallelism have shown that the application of the criterion of the gene as a unit of function must be viewed with caution. When the criteria of crossing over and function are in conflict, it seems most reasonable to regard the gene as the smaller unit within which no subdivision by crossing over occurs. The function of the smaller unit may not be independent of its neighboring units, as in the case of position pseudoalleles.

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Lewis, E. B., 1951, "Pseudoallelism and Gene Evolution." Cold Spring Harbor Symposium on Quantitative Biology. Volume XVI. pp. 159-174.



## GENES AND ENZYMES

Abstracted by Roy Sachs and David R. Stadler

The preceding paper presented the genetic evidence for considering the existence of genes as the discrete units of heredity; the nature of gene action in determining heritable differences by entering into the biochemical processes of organisms is the subject of this paper.

In 1902, two years after the rediscovery of Mendel's investigations and early in the development of enzymology, Cuenot suggested that inherited differences in coat color in mice might be related to the amount of tyrosinase in the skin.

The first intensive investigations which eventually led to the concept of the gene as a functional unit in biochemical processes were those relating to the abnormalities of the tyrosine - phenylalanine metabolism in man. Alcaptonuria, an early known disease, was the first such metabolic error subjected to both biochemical and genetical study.

A symptom of alcaptonuria is blackening of the urine, and Garrod, in his book, "Inborn Errors of Metabolism", published in 1909, had accumulated evidence from earlier investigations which indicated that the blackening of the urine was due to the urinary excretion of homogentisic acid (alcapton; 2,5 dihydroxyphenylacetic acid), a substance which accumulated in alcaptonurics. Garrod related these observations to those of Bateson and Punnet which indicated that alcaptonuria behaved in inheritance in the same fashion as a simple Mendelian recessive trait and Garrod suggested the dependence of the biochemical constitution of an organism upon its genetic makeup.

In 1914 Gross reported the presence of an enzyme in the blood serum of normal individuals which catalyzed the breakdown of homogentisic acid and, further, said he could not detect this enzyme in the sera of alcaptonurics.

Implicit in these investigations was the assumption that a single gene is involved in determining the presence of a specific enzyme.

Also in the year 1914 Onslow made the first study of biochemical changes involved in flower - color variation and noted that the intraspecific variation of flower pigments provided an opportunity to relate gene activity to the structure of the pigments involved. During the next two decades investigations into the chemistry of flower pigments provided the analytic tools necessary for biochemical and genetic studies as suggested by Onslow. When the chemical and genetic data were correlated for anthocyanin pigment variation, it was observed that single gene substitutions were accompanied by chemical modifications in the anthocyanins, the nature of which indicated a simple one-step chemical reaction.

Investigations into the nature and mode of synthesis of eye pigments in the fruit fly, *Drosophila melanogaster*, led to the discovery that the single gene change from wild type to vermilion was accompanied by loss of the ability to synthesize kynurenine, a tryptophan derivative.

In white clover and other legumes it is known that there are inter-plant differences in cyanogeneticglucoside content; these compounds under certain conditions may liberate HCN and thereby prove toxic to livestock. It has been shown that strains may differ genetically both in their ability to synthesize these compounds and in the presence of

the hydrolytic enzyme - each is dependent on the dominant allele of a specific gene and the two genes are inherited independently.

The further development of biochemical genetics awaited certain advances in both genetics and biochemistry, and among these were the discovery of the mutagenic action of high-energy radiations, the development of enzymology, the concept of one-step chemical reactions composing metabolic systems, and advances in the field of nutrition.

The discovery of nutritional mutants of the fungus, Neurospora crassa, by Beadle and Tatum in 1941 provided an organism which was well suited for both biochemical and genetic studies, and provided the opportunity to test further the one gene - one enzyme hypothesis which had been implied in previous investigations. The wild-type Neurospora is capable of synthesizing all of its cellular constituents except Biotin from simple carbohydrates and inorganic salts. It was possible for Beadle and Tatum to alter the nutritional requirements of the wild type by inducing gene mutations (by x-ray and UV treatment), which led to the failure of certain biochemical syntheses to occur in the mutants; the resulting mutant strains are unable to grow in the absence of the product whose synthesis has been abolished.

Since Neurospora has a sexual mode of reproduction the mutants can be analyzed genetically, and, by asexual propagation, large quantities of genetically homogeneous material can be obtained for biochemical study.

Other micro-organisms (particularly Escherichia coli, a bacterium, and Saccharomyces cerevisiae, a yeast) have been subjected to both biochemical and genetic study and the following will describe a few investigations with micro-organisms which have further elucidated the nature of gene action.

The studies of the chemical constitution of mutant microorganisms can be divided into two groups: those which have shown quantitative changes in the enzyme complement of the mutant strain (absence or reduced amount of some enzyme present in the wild strains), and those which have shown qualitative alterations in some protein found in both the mutant and the wild type.

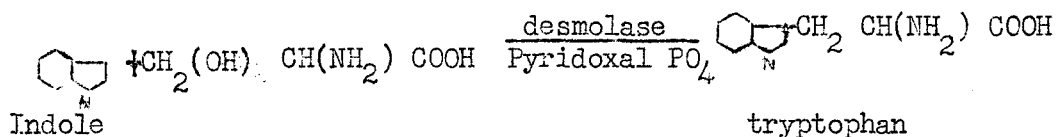
#### I. Mutations Involving Quantitative Alteration of Proteins

Tryptophan synthesis in *Neurospora* was first studied by Tatum and Bonner in 1943. It was demonstrated for the first time that as a result of genetic blocking there was an accumulation of a metabolic intermediate. A mutant strain which lacks the ability to convert anthranilic acid to indole and thence to tryptophan excretes anthranilic acid in the growth medium; this is true when sufficient tryptophan for growth is supplied to the medium, indicating that there is some "automatic" reaction producing the intermediate, which accumulates for lack of an effective mechanism to remove it. This observation coupled with the fact that anthranilic acid promotes growth in other tryptophan-requiring mutants indicates that the compound is a natural precursor of tryptophan.

Precursor accumulation has been a fairly common observation among other *Neurospora* mutants and many of these compounds have been isolated and identified, aiding in determining biosynthetic pathways.

It has been shown that tryptophan is formed from indole by condensation of the latter with a molecule of serine. The enzyme responsible for this reaction, tryptophan desmolase, has been isolated from wild-type *Neurospora*; pyridoxal phosphate was required as a cofactor for the

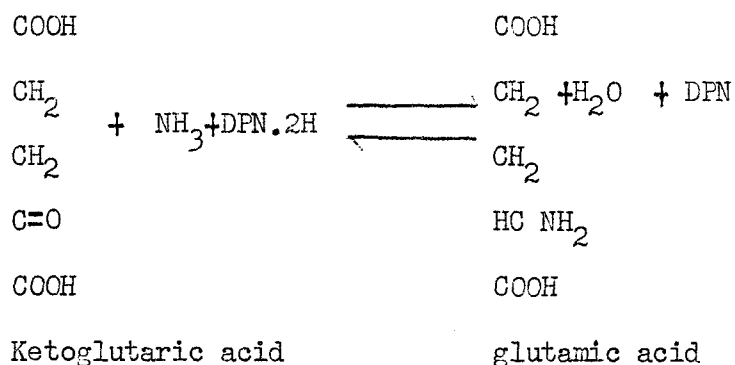
reaction. Tryptophan-requiring mutants blocked at the Condensing step have been analyzed for the desmolase. No enzyme activity was found; whereas mutants requiring tryptophan but blocked at other stages in the synthesis were reported to contain the same activity, with respect to the desmolase, as did the wild type. To the limit of the methods of analysis employed, it can be said that, in this case, a single gene mutation was directly responsible for the absence of an enzyme normally present in wild-type organisms.



#### GLUTAMIC ACID DEHYDROGENASE

Certain mutant strains of *Neurospora* are unable to synthesize the amino groups for a wide range of amino acids from  $\text{NH}_3$ , yet these strains are analyzable as single gene mutants. All previous evidence indicated that a single gene mutation produced a change in but one enzyme.

If a one gene -- one enzyme hypothesis were to hold in this case, then it is necessary to postulate that the enzyme affected by this gene mutation enters into some basic chemical reaction which controls the synthesis of a number of amino acids; by implication there is, then, one primary reaction by which amino groups for these amino acids are made from ammonia. The suggestion was put forward that this reaction might be the reductive amination of  $\alpha$ -ketoglutaric acid to glutamic acid; a reaction catalyzed by glutamic acid dehydrogenase and reduced diphosphopyridine nucleotide as cofactor.



The glutamic acid, thus synthesized, serves in numerous transaminase reactions as an amino group donor. Fincham, in investigations with these mutants, found that glutamic acid, aspartic acid, and alanine enabled the mutant strains to grow as well as the wild type. He later found that the mutant strains lacked glutamic acid dehydrogenase and were unable to fix  $\text{NH}_3$  in the form of amino groups; if the mutants were given a utilizable source of  $\text{NH}_2$  groups (as present in glutamic acid) then other amino acids could be synthesized by transamination reactions. This is another example in which a single gene substitution produced strains which were found to be lacking one enzyme.

Much biochemical work has been done on mutant strains of coli bacteria which differ from the wild type in that they require specific growth factors added to the minimal medium. In certain cases these strains have been shown to lack an enzyme or enzyme system which is present in wild type. Unfortunately, the process of bacterial reproduction with regard to the exchange and passage of genetic material is not yet well understood. These mutant strains which have been defined chemically are not amenable to genetic analysis, so it has been impossible to say definitely that they differ from wild type by only a single gene, although this seems probable.

Wild strains of E. coli have been shown to contain an extractable enzyme system which decarboxylates  $\alpha$ - $\epsilon$ -diamino pimelic acid to lysine and carbon dioxide. Extracts of three lysine-requiring strains show no detectable activity of this system, and all three accumulate diamino pimelic acid in the medium. The evidence suggests that diamino pimelic acid is a normal precursor of lysine in E. coli and that these mutants differ from wild type by the absence of the active enzyme for this decarboxylation.

Vogel has studied two ornithine-requiring mutants of E. coli. These are shown to be blocked at different steps in the synthesis of ornithine by the fact that mutant "a" accumulates and excretes an intermediate which can be utilized for growth in place of ornithine by mutant "b" (this indicates that mutant "b" is blocked at an earlier step in the synthesis). The excreted intermediate has been isolated chromatographically and identified as N  $\alpha$ -acetyl-L-ornithine. A cell-free enzyme system capable of converting this substance to L-ornithine has been extracted from wild-type bacteria and from mutant "b", but extracts of mutant "a" show none of this activity.

A mutant of coli which has been studied by Maas requires pantothenic acid for growth. This substance is synthesized in wild strains by the coupling of  $\beta$ -alanine and pantoic acid, but the mutant fails to grow when supplied with these compounds in place of pantothenate. Cell-free preparations from wild strains give active coupling of  $\beta$ -alanine and pantoic acid, but extracts of the mutant fail to show any activity of this coupling enzyme.

## II. Mutations Involving Qualitative Alteration of Proteins

There have, to date, been only a very few cases in which it has been clearly demonstrated that gene mutation has resulted in the production of a protein in a form which is qualitatively different from that produced by the wild type. Pauling, Itano, and their collaborators have shown that the occurrence of sickle-cell anemia, a heritable blood disease, is correlated with hemoglobin differing in its physical chemical properties from that of normal individuals. Electrophoretic studies show that red blood cells of persons suffering from the disease contain an altered form of the hemoglobin molecule. (It is suggested that the difference results from altered number of ionizable groups on the globin portion of the molecule.) Measurements of solubility in phosphate buffer have shown the sickle cell hemoglobin to be much less soluble than normal hemoglobin. Studies of inheritance of the disease indicate that it can be determined by a single gene difference from normal individuals.

Maas and Davis, who showed that the pantothenic-acid-requiring mutant of E. coli lacked the enzyme for coupling  $\beta$ -alanine to pantoic acid, have also studied another mutant which required pantothenic acid for growth only at high temperatures. Below 30°C. it grows on minimal medium like wild type. They have shown that this strain, when grown at a low temperature, produces the coupling enzyme. But this enzyme differs from that produced by wild type in being extremely sensitive to inactivation by heat. The enzyme in extracts of wild type is completely stable at 35°C., while extracts from the mutant are largely inactivated by treatment of one hour at 30°C. Heat treatment of a mixture of the two extracts gives residual activity equal to the sum of the activities of control extracts heated separately. This indicates that the



difference in heat stability of mutant and wild type results from a change in the enzyme molecule itself and not from the presence of some other substance which sensitizes the enzyme to heat inactivation (or the absence of a normal inhibitor of temperature inactivation). It is unfortunate that present methods do not enable us to determine whether the strain with an absolute pantothenate requirement results from mutation at the same genetic locus as the one causing this requirement at high temperatures.

The production of tyrosinase by *Neurospora* has been studied by Horowitz and Fling. Tyrosinase catalyzes the oxidation of phenolic compounds to the corresponding quinones and is involved in the production of melanins, the black plant and animal pigments, from tyrosine. Tyrosinase may not be an essential enzyme for *Neurospora* because a number of strains have been studied which produce no detectable tyrosinase at 35°C., yet growth appears to be perfectly normal at this temperature. Further study of the temperature relations of tyrosinase has shown that the strains of *Neurospora* used in this study can be divided into two groups according to the heat sensitivity of their tyrosinases. One group produces tyrosinase which is relatively stable to heat treatment (called T<sup>S</sup>), while the other type is much more temperature labile (T<sup>L</sup>). Extracts of T<sup>S</sup> strains lose half of their tyrosinase activity if held at 59°C. for 40 minutes, while T<sup>L</sup> extracts drop to one-half of their original activity in three minutes at this temperature.

When a T<sup>S</sup> strain of *Neurospora* is crossed to a T<sup>L</sup> strain, half of the progeny are like each parental strain as regards temperature sensitivity of tyrosinase. From every ascus produced in the cross, two of

the spore pairs give  $T^S$  strains and two give  $T^L$ . Therefore it is concluded that this character is controlled by a single genetic factor.

It is of interest to know more precisely what is the difference in chemical constitution between  $T^S$  and  $T^L$  strains which cause the tyrosinase activity of one to show more resistance to high temperatures than that of the other. Is it a difference in the enzyme molecule itself? Or is it a difference in amount or quality of some other constituent of the extract which influences the action of heat on the protein molecule? Three separate lines of evidence indicate that the difference lies in the tyrosinase itself.

*Neurospora* tyrosinase has been extracted and partially purified from the crude extracts by fractional precipitation in ammonium sulfate. This method has yielded a 44-fold purification of the temperature-stable tyrosinase and a 13-fold purification of the labile form. When heat-treated after purification, they still show at least as great a difference in temperature sensitivity as in the crude extracts. This would not have been the case if other materials in the extracts had been responsible for the difference.

Dilutions, up to 32-fold, do not change the rate of inactivation of enzyme preparations in heat. This indicates that the inactivation is a mono molecular reaction, dependent on the concentration only of the enzyme itself. If some other constituent were also required for the inactivation process, then dilution would cause a drop in rate.

Mixing experiments show that the rate of temperature inactivation of tyrosinase of one type is not affected by the presence of the extract of a strain of the other type. The residual activity after such treatment

is always equal to the sum of the activities of the two component extracts heated separately. Again, a change in the enzyme molecule itself appears to be responsible for the observed difference in temperature sensitivity. The evidence seems clear in this case that a change in a single gene results in the qualitative alteration of a protein produced by the organism.

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## ANALYSIS OF BIOSYNTHETIC PATHWAYS BY GENETIC EXPERIMENTS

Abstracted by Sterling Emerson

Examples of interactions between genes affecting biosyntheses of metabolites in *Neurospora* were reviewed, together with interpretations based on the idea that each gene has a single primary function in cell physiology.

The accumulation of imidazole side-products in the presence of pairs of mutants, each of which interrupts the synthesis of histidine at a different step, was cited as an example of methods used in studying the combined effects of different genes, and of the additional evidence that is obtained thereby.

Mutants which differ from the ordinary tryptophaneless mutants and nicotinicless mutants in that they respond to either tryptophane or nicotinamide were interpreted as having no block to the synthesis of these compounds, but having an enhanced requirement for nicotinamide. Any of the precursors of nicotinic acid (including tryptophane and its precursors) could satisfy the additional requirement for nicotinamide, provided each biochemical step intervening between the precursor and the final product responds to increased substrate concentration by a sufficient increase in the rate of the reaction involved. Genes which tend to limit the rates of one or another of the intervening reactions to approximately those occurring in wild-type strains should, when combined with the mutant under discussion, modify its effect by producing the appearance of a complete block at the limiting step, thus accounting

for the effects of modifying genes reported by Haskins.

Complex interactions between genes apparently primarily involved in the biosyntheses of pyrimidines, lysine, or the related group of amino acids consisting of proline, ornithine, citrulline and arginine were interpreted by the following assumptions:

1) Pyrimidineless-3, a mutant apparently blocking an intermediate step in pyrimidine synthesis, is supposed to have a slight retarding influence on a number of related chemical reactions, i.e., those either adding a carboxyl group to an omega-amino group of an alpha-amino acid, or aminating an omega-carboxyl group of similar compounds. The implicated step in pyrimidine synthesis must be especially sensitive in this respect. The effects on other, similar reactions would account for (a) the difficulty of substituting alpha-amino adipic acid for lysine by certain lysineless mutants in the presence of pyrimidineless-3, and (b) the difficulty in using citrulline by certain arginineless mutants when pyrimidineless-3 is present.

2) The suppressor of pyrimidineless-3 is similarly supposed to have a rather general effect in cell physiology, tending to increase the rates of oxidation in a number of different reactions. It counteracts the effect of pyrimidineless-3 by increasing the rate of the limiting reaction through an increased concentration of the substrate donating the carboxyl group to the reaction -- in the absence of the suppressor the group involved tends to be present in the reduced, aldehydic form. Other interactions accounted for are (a) the interference by the suppressor in the utilization of alpha-amino adipic by lysineless mutants through decrease in the rate of reduction of the

omega-carboxyl group, (b) the additive effects of the suppressor and pyrimidineless-3 on alpha-aminoadipic utilization since they influence different reactions, (c) the interference with the utilization of ornithine by certain arginineless mutants in the presence of the suppressor because ornithine tends to be oxidized to glutamic semi-aldehyde.

3) Alpha-aminoadipic acid is supposed to be the donor of the carboxyl group in the reaction in pyrimidine synthesis affected by pyrimidineless-3. This accounts for (a) the accumulation of pyrimidine-like substances by certain lysineless mutants by shunting accumulated lysine precursors into the pyrimidine pathway, (b) the suppression of mutants requiring any one of proline, citrulline, ornithine or arginine by the suppressor of pyrimidineless-3, since, if alpha-aminoadipic acid is the precursor in pyrimidine synthesis, a product of this synthesis must be delta-hydroxy-norvaline, which is known also to be utilized by the proline mutants and which must increase in the presence of the suppressor.

Mutants having a general effect on a number of related biochemical reactions supposedly have one primary effect which is in turn responsible for the diverse effects observed. For example, the suppressor of pyrimidineless-3 might influence some one reaction in such a way that a particular co-enzyme tended to be left largely in the oxidized form. The presence of an excess of the oxidized co-enzyme would tend to push other reactions using the same co-enzyme in the oxidative direction. The same effect would result if the primary reaction influenced the concentration of sulfhydryl groups.

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# CYTOPLASMIC INHERITANCE

Abstracted by Barbara M. Gowdridge and Burke H. Judd

In sexually reproducing organisms it is usual for the female parent to contribute a nucleus and the cytoplasm to the zygote while the male contributes a nucleus and little if any cytoplasm. In general the manner in which a genetic factor is inherited is independent of the parent through which it is introduced. But there are factors which are inherited only or largely from the female parent and are associated with the cytoplasm.

Some examples of cytoplasmic inheritance are given in Table 1. The earlier known instances (1 - 4) involved variegation in higher plants. When the female parent is variegated and the male normal, the progeny may be white or green or variegated. In the reciprocal cross the progeny are all green. Such effects have been associated with chloroplasts supplied by the maternal cytoplasm. One view is that two types of chloroplasts, white and green, occur in a variegated plant and varying proportions of these types may be passed on to the offspring.

Carbon dioxide sensitivity in *Drosophila* and the killer character in *Paramecium* are similar in that the inheritance is associated with large complex particles in the cytoplasm comparable with viruses. They may be transmitted and are infective.



TABLE I  
SOME EXAMPLES OF CYTOPLASMIC INHERITANCE

ORGANISM	Phenotypic Expressions	OBSERVERS
1. <i>Mirabilis</i>	Variegation	Correns (1909)
2. <i>Pelargonium</i>	Variegation	Baur (1909)
3. <i>Epilobium</i>	Variegation -Pollen Sterility Morphology -Enzyme differences	Michaelis (1923)
4. Maize	Pollen sterility, Variegation	Rhoades (1933)
5. <i>Drosophila</i>	CO <sub>2</sub> Sensitivity	L'Hertier and Teissier (1937)
6. <i>Paramecium</i>	Killer substances, Antigenes, Mating types	Sonneborn (1943)
7. <i>Puccinia</i>	Pathogenicity	Newton and Johnson (1932)
8. <i>Saccharomyces</i>	Small colonies, Cytochrome Differences, Other enzymes	Ephrussi (1949)
9. <i>Neurospora</i>	Growth rates, -Cytochrome differences, - Other compo- sition differences.	Mitchell and Mitchell (1951)

In yeast again there are particles, perhaps equivalent to a mitochondria in animal tissue; thus it is possible to assume that the inheritance of a small colony characteristic is associated with the particles and that they are redistributed at cell division.

There are, however, a number of examples of cytoplasmic inheritance which have not been shown to be associated with particles. Michaelis does not advance such an explanation for the many differences which he has shown to be inherited cytoplasmically in *Epilobium*.

# CYTOPLASMIC INHERITANCE IN NEUROSPORA

Some eight strains of Neurospora are known which exhibit maternal inheritance. Of these "poky" (mi-1) and mi-3 are distinct and the other six resemble mi-3 insofar as they have been analyzed; all grow more slowly than wild type. Under particular conditions, when grown in liquid minimal medium, wild type reaches a maximum dry weight of nearly 100 mg. in four days; (mi-1) poky about 20 mg. in seven and one-half days and mi-3 about 60 mg. in six days.

Differences in the cytochrome systems of these strains have been revealed by spectroscopic examination following treatment with sodium hydrosulphite. (Table 2). The absorption spectrum of wild-type Neurospora resembles that of liver tissue and shows bands corresponding to cytochromes c, b and a+a<sub>3</sub>. The most striking difference from this in both poky and mi-3 is the large increase in the amount of cytochrome c.

TABLE II

## CYTOCHROMES IN NEUROSPORA STRAINS

	Cytochrome Spectra			
	<u>c</u>	<u>b<sub>1</sub></u>	<u>b</u>	<u>a+a<sub>3</sub></u>
Wild type	+	-	+	+
mi - 3	+			
	5 x wild type	-	+	-
poky (mi-1)	+	-	trace*	trace*
	15 x wild type			
C 115	+	-	+	-
	5 x wild type		<wild	
C 117	-	+	+	-
			>wild type	
C 115 in poky	+	-	-	-
	> poky			
C 115 in mi-3	+	+	+	-
	> poky			
C 117 in mi-3	-	+	+	-
			>wild type	

\*Detectable at the temperature of liquid nitrogen.

In addition to the cytoplasmically inherited characters, there are also, certain nuclear genes which affect the cytochrome systems and the growth rates of the strains concerned, as for example strains C115 and C117. The cytochrome spectra of these strains are indicated in Table II. It will be seen that they are different from each other and from the spectra of both poky and mi-3. Table 2 also shows the spectra of other strains obtained from crosses designed to introduce the nuclear genes into poky or mi-3 cytoplasm. (In the case of C117 in poky the growth is so slow that analysis has not been done.) With C115 in poky as an example it can be seen that the spectrum differs from that of both C115 and poky, nor is it the sum of these. C115 in mi-3 gives similar results.

TABLE III  
COMPOSITION OF WILD TYPE AND POKY NEUROSPORA

Component	Relative Concentration <u>poky</u> wild	Cellular Fraction Affected
Nucleic Acids		
DNA	1.3	Nuclei
RNA	1.0	----
Extractable Polysaccharides	1.0 (0.25, 1.3)	Particles, Soluble
Riboflavin (FAD)	2.0	Particles, Soluble
Niacin	2.0	Soluble
Cytochrome <u>c</u>	15.	Particles, Soluble
Cytochromes <u>a</u> + <u>a</u> <sub>2</sub> and <u>b</u>	<0.02	Particles
Succinic Dehydrogenase	1.0	Particles
Catalase	1.0	Soluble
Cytochromase	>100.	Particles
Protein X	<0.2	Cell Debris
Pyrophosphatase	1.5	Soluble
Other Enzymes (Differences of doubtful significance)		
Metaphosphatase, acid and alkaline phosphatases, tryptophane desmolase, phosphogluconic dehydrogenase, amylase, uricase, invertase, transaminases		

Table III shows that poky differs from wild type in components other than the cytochrome system. There is a slight excess of DNA, of uncertain significance, in poky compared with wild type while the RNA is equal. This does not necessarily mean that the distribution of RNA among the cellular components is the same in the two strains and limited evidence indicates that they are not. The data regarding the extractable polysaccharides are interesting in this connection. Here also the total amount is the same in both strains but there is a deficiency in the poky particles and an excess in the soluble cellular fraction. Another point of significance in Table III is that poky shows an excess of cytochrome c in the soluble fraction as well as in the particles.

It is of special interest that poky contains cytochromase, an entirely new enzyme system which destroys cytochromes and which is not detected in wild type. This enzyme system is present in C117 as a result of a single gene difference. The system does not act on other heme compounds. Its action is believed to be in part proteolytic and this is followed by oxidation of the heme to a biliverdin-like substance. An inhibitor of cytochromase has been shown to be present in poky and the presence of so much cytochrome c in spite of the occurrence of cytochromase may be due to its action. There is, however, an indication of incomplete inhibition here since old cultures of poky turn the medium slightly green.

Since poky grows much more slowly than wild type and also shows differences in its cytochrome system it is not surprising to find that the respiratory rate is about two and a half times greater in wild type than in poky. Table IV indicates the rates of O<sub>2</sub> uptake and the effect of azide on it in both strains.

TABLE IV  
RESPIRATION IN WILD TYPE AND POKY NEUROSPORA

			<u>+0.001M Azide</u>	<u>% Inhibition</u>
Intact Mycelium	<u>W.T.</u>	70	34	52
( $\mu$ l O <sub>2</sub> /hr./mg.)	<u>po</u>	30	33	-10
Cell-free Extracts	<u>W.T.</u>	110	52	53
( $\mu$ l O <sub>2</sub> /hr./mg.)	<u>po</u>	20	26	-30

TABLE V  
INTERACTIONS OF CELLULAR FRACTIONS FROM WILD-TYPE AND POKY NEUROSPORA -  
RESPIRATION

W= wild type; po= poky; P= particles; S= soluble fraction.

Oxygen Uptake ( $\mu$  liters/hr.)

		<u>+0.001M Azide</u>	<u>% Inhibition</u>
WP + WS	150	95	30
<u>poP</u> + <u>poS</u>	90	35	5
WP + <u>poS</u>	221	31	63
<u>poP</u> + WS	35	20	43

The data in Table IV might indicate that there are at least two respiratory systems in wild type and that in poky one of them (azide-sensitive) is not functioning. To test this the respiratory rates of combinations of particles and supernates from the two strains were determined. These data are given in Table V and they indicate that the respiratory differences in the two strains cannot be interpreted on the simple basis suggested.

Attention should be drawn to a number of final points of interest. Are the characteristics of these strains caused by infective particles in the cells? In crosses where poky is used as conidial parent there is no evidence that the protoperithecial parent is infected from it. Also preliminary immunological studies give no evidence that foreign particles are present.

It is clearly an oversimplification to think of poky as differing from wild type merely in the nature of its particles. We have seen that there are also many differences in the soluble fractions. It may be that the same potentialities exist in both strains and that the difference is in the intensities of various reactions in the two. It is apparent that there are two stable metabolic states existing here and it is possible that any number of factors acting singly or together might cause a shift from one to the other. The analysis of such factors, both internal or external to the cell, is one problem outstanding; but it should be remembered that in any analysis it may be difficult to distinguish between the cause and the effects of a change of metabolic state. It is also important to keep in mind that the causative agent may not be present at the time the effect is expressed.

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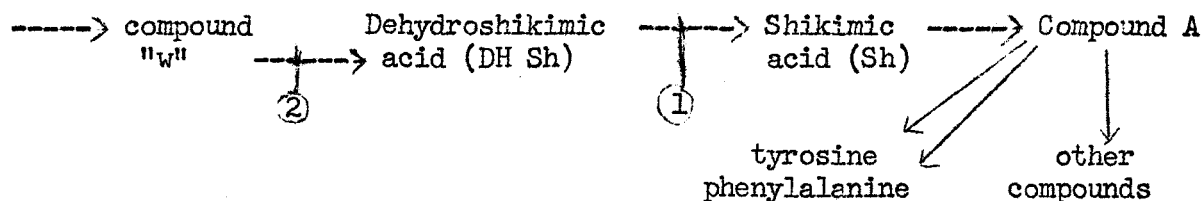
# MUTATIONS AND PHYSIOLOGICAL VARIATIONS IN BACTERIA

Abstracted by Joseph T. Holden and Richard S. Schweet,  
and Paul O.P. Ts'ao

Bacterial genetics has recently made rapid progress. In many instances, however, basic genetic phenomena have been found to be so closely integrated with complex physiological events as to be indistinguishable. Thus, a discussion of the interrelation between physiological and genetic factors is required as a basis for the understanding of bacterial genetics.

Any genetic change in bacteria and other organisms will be recognized from its phenotypic effect. Since a complicated system of enzymes acts between the gene and its final effect, a change in the gene will be detected through the change in the constitution or function of the enzymes. A limited analysis of the interrelations of some enzyme systems which have been studied in bacteria will be presented preparatory to a discussion of bacterial variation.

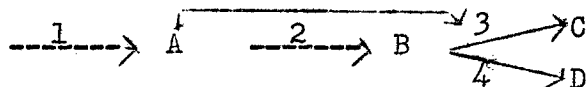
The synthesis of aromatic compounds in E. coli has been shown by B.D. Davis and co-workers as follows:



Two metabolic interactions have been observed within this sequence. A competition exists between Shikimic acid (Sh) and its precursor (DHSh) for the enzyme which acts on Sh. A mutant blocked at ① will not grow even with the addition of Sh unless tyrosine and phenylalanine are also

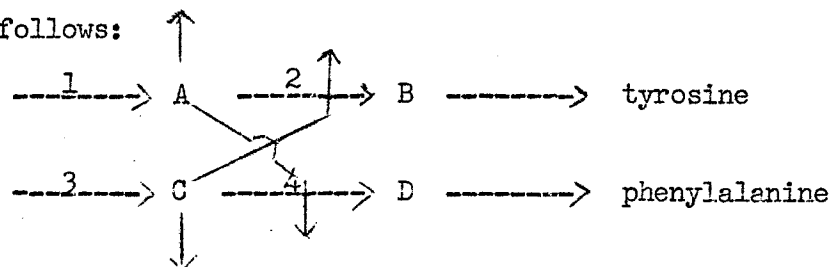
supplied. Mutants blocked at 1 and 2 (no accumulation of DHSh) grow with Sh in the absence of the two amino acids, unless DHSh is added to the medium when they are again required.

A more generalized pathway is the following:



If product  $[A]$  is considered to be somewhat inhibitory to reaction 3, a partial genetic block of reaction 2 (resulting in accumulation of  $[A]$ ) will have little effect on the production of compound  $[D]$ , since more  $[B]$  will be available for reaction 4 due to inhibition of reaction 3. A similar self-compensatory mechanism with respect to compound  $[C]$  would arise in the case of a partial block of reaction 1.

Another example is found in the pathways leading to the synthesis of tyrosine and phenylalanine. It appears that a precursor of phenylalanine inhibits tyrosine synthesis, and that a precursor of tyrosine inhibits phenylalanine synthesis. The interaction can be visualized as follows:



A partial block of reaction 1 decreases the concentration of  $[A]$ , thus relieving the inhibition of reaction 4. Therefore  $[D]$  increases and  $[C]$  decreases, thus relieving the inhibition of reaction 2 and increasing the concentration of  $[B]$ . Such a system is self-regulatory (at the expense of the side-reactions indicated by the arrows).



It can be seen from these examples that the effect of a single partial genetic block may be observed at several points not directly related to the primary block, or may not be observed. It is also possible that the examples given are only part of the total interrelationships which exist in the cell, so that the effect of the single block may spread out over a wide network of reactions. Changes in the concentration of an intermediate may ultimately affect the synthesis of enzymes involved in the pathway. One can visualize that the observable changes may vary in a continuous way, or alternatively, that no change may be noted until at some critical concentration (of enzyme or product) a switch to a new stable state of the system would occur.

To summarize, by this interpretation an observed change in some bacterial activity would be related to the primary block in some complex way involving changes in the amount of various substrates and the activity of various enzymes. A unique change in the system could simultaneously affect several activities of the cell; or the change of a single activity could be the consequence of several different changes in the system.

The next point to be considered in this analysis will be the factors which affect the concentration of the enzymes themselves, an important variable in the system.

Enzymatic adaptation is a well known case in which the concentration of an enzyme in the cell is found to increase. In bacteria we define this process as one by which a population of cells unable to carry out a certain reaction temporarily acquires this ability as the consequence of contact with a substance similar to or identical with the substrate of the enzyme. All the cells of the population simultaneously undergo this change, which persists as long as the cells are in contact with the substance which produces the adaptation, the stimulator.

The adaptation to lactose fermentation in Enterobacteriaceae has been extensively investigated and has been shown to consist in a thousandfold increase in the concentration of the enzyme  $\beta$ -D-galactosidase, which initially hydrolyses lactose. This has been proven by measurements of enzyme activity in extracts and of enzyme protein by precipitation with specific antiserum.

The hypothesis that the increase of enzyme concentration in the presence of lactose is simply a substrate-induced change of a non-functional into a functional enzyme is not supported by the observations of Monod and of Lester and Bonner. They found that many compounds possessing the galactosidic radical (most of them not substrates and some having no affinity for the enzyme) can stimulate increases in enzyme concentration. Studies of Swanson and Giese indicate that the increase in enzyme concentration occurs without the participation of the enzyme. UV irradiation of cells before exposure to stimulator prevented adaptation while  $\beta$ -galactosidase itself is unaffected by such treatment. The action spectrum of the UV suggests that the enzyme-forming systems contain nucleic acid. The enzyme however does not contain nucleic acid. Spiegelman, studying the loss of adaptation in yeast cells, has concluded that adaptive enzymes are produced by granules present in the cells in relatively small numbers. The stimulator may act on these granules. The idea of enzyme-controlling granules which contain nucleic acid is in agreement with other data. The experiments of Pollock on penicillinase adaptation indicate that the stimulator (in this case penicillin), wherever it does act, is found in the cell in extremely minute amounts.

Another question which has concerned investigators is whether the net increase in enzyme concentration results from increased production or decreased breakdown. Cohn and Torriani suggest that an enzyme precursor is converted to enzyme during adaptation. Immunological experiments show that adaptation is accompanied by some decrease in concentration of a protein serologically related to  $\beta$ -galactosidase which is present in unadapted cells.

Halvorson and Spiegelman have found that the presence of an amino acid analog prevents adaptation and that incorporation of free amino acids (presumably into protein) was blocked. They have interpreted these results to mean that formation of adaptive enzyme proceeds from the free amino acid pool, rather than a precursor protein.

It is possible that the real mechanism involves some combination of these opposing theories; however, the data do not permit a decision as yet.

Recently, Vogel and Davis have presented evidence which suggests that the concentration of the constitutive enzymes (those which appear to be normal cell constituents) is also controlled by stimulators. If these ideas are correct, we see that possibly the synthesis of enzymes may generally be regulated by simple compounds, which resemble their substrates. These may be exogenous or produced by other enzymes in the cell. The functional network we have originally considered should now be extended to include enzyme-controlling granules.

If the enzyme-controlling granules were self-reproducing, the network would display all the characteristics of a living organism. A bacterial cell is produced by fission from another cell, and in this way begins

its life with a complete network of enzyme-controlling granules, enzymes and substrates. Since the system tends to maintain the original stable state, the cell would display a permanent phenotype, but could undergo permanent variation through changes or disappearance of some of the enzyme-controlling granules. The question arises whether bacterial variation can be accounted for in terms of such a "cytoplasmic" system, or whether a control system, at a level comparable to the genetic system of higher organisms, functions in this case.

An answer to this question requires an analysis of the process of bacterial variation. Common examples of bacterial variation are the change from smooth to rough, loss or gain of ability to synthesize certain compounds or ferment sugars, increase or decrease of resistance to bacteriophages or drugs, and so on. Unfortunately, in most cases such changes can be detected only in the presence of the agent which is to be acted on by the altered cell. The question arises, then: does the agent simply detect the variants, or does it participate in their production? A decisive answer to this question is required, since the previous discussion showed that the concentration of enzymes in the cells is under the control of simple substances.

Proof of the spontaneity of such changes, independent of external influences, rests on the fact that variant cells can be shown to occur in clones and not uniformly distributed throughout the culture. If phage-resistant cells, for example, arise spontaneously, independent of the exposure to phage which is used to detect these cells, they would arise at all times during the development of the cultures, and thus at the moment of test the resistant variants will have developed to varying sized clones of resistant cells. If, on the other hand,

resistant cells are produced by phage action, they would be found distributed throughout the culture and not in clones. Luria and Delbrück<sup>"</sup> have conclusively shown the presence of variant clones by a statistical technique.

More direct methods for the detection of clones have been described by Newcombe and by Lederberg. In both cases, conclusive evidence was obtained to show the occurrence of spontaneous variation to phage resistance, independent of the testing agent. A limiting factor in all these experiments, however, is that only changes in resistance to an unfavorable agent or to gain of a new synthetic or fermentative capacity have been studied. As yet no experiments have been designed to detect a spontaneous loss of synthetic ability or a greater sensitivity to damaging agents.

The type of spontaneous variability which has been discussed is essentially different from enzymatic adaptation in the following ways:

<u>Spontaneous variability</u>	<u>Enzymatic adaptation</u>
a. Spontaneous	a. Controlled by environment
b. Heritable	b. Temporary
c. Affects a limited number of cells	c. Mass phenomenon

In view of these differences, it is unlikely that the spontaneous variation is based on the same type of enzyme control which governs enzymatic adaptation. These facts necessitate consideration of the possibility of enzyme control at a higher (genetic) level.

The similarity between spontaneous variations occurring in bacteria and those occurring in higher organisms of known genetic constitution through mutation suggests that such changes in bacteria are also mutations. This can be proved by the use of genetic techniques, namely, by demonstrating the occurrence of genetic recombination among different characters. While this has been achieved recently in bacteria, unexpected difficulties have been encountered which complicate the interpretation of the data. Dr. Vogt will discuss this aspect of the problem.

Nonetheless, spontaneous changes could in all probability be identified with mutations even in the absence of recombination, if it could be demonstrated that they originate in the nuclear apparatus of the bacteria. Witkin has attempted to provide such evidence. Bacteria are known to possess special bodies containing DNA. These bodies behave, during cell division, in a way very similar to nuclei of higher cells, and can be considered homologous structures. In E. coli every cell has more than one nucleus. Thus, if a mutation is induced in a cell, generally only one nucleus will be affected, due to the low frequency of mutation. The cell will not be phenotypically changed immediately, but the effect of the mutation will appear after several divisions when a cell arises whose total nuclear apparatus derives from the nucleus where the mutation took place (segregation lag). All other cells will contain some unchanged nuclei and will therefore appear unchanged. The original cell in which a mutation occurred will give rise to a mixed clone, and if the colonial type of the mutant differs from the parent on solid media a sectorized colony will be formed.

Nuclear segregation evidence of this type has been obtained by Witkin with E. coli, lactose-positive ( $\text{lac}^+$ ) cells. These were irradiated with UV and then plated on EMB lactose agar where  $\text{lac}^+$  are red and  $\text{lac}^-$  are pink colonies.  $\text{Lac}^-$  mutations appeared as complete colonies and as sectors. The ratio of sectoried to complete colonies was higher when young bacteria, containing more nuclei per cell, were used as compared with old bacteria. More convincing evidence for the existence of nuclear segregation has been obtained from a technique suggested by Visconti. After UV irradiation, the cells were permitted to grow for a few generations and then sprayed with phage. Thus double mutants ( $\text{lac}^-$  and phage resistant) would be detected. If nuclear segregation took place sectoried colonies should not occur, since these would require that one nucleus in the cell be the double mutant ( $\text{lac}^-$ , phage resistant) and another nucleus in the same cell be the single mutant (phage resistant) an improbable event. The results supported the concept of nuclear segregation.

In conclusion, we may picture the appearance of a mutation in a multi-nucleate bacterium as occurring by a series of steps. First, a change in one nucleus. The cell, transformed into a heterocaryon, divides and after several divisions (segregation lag) one cell is produced which is pure for the mutated gene. As a result there will be some change in the enzyme-controlling granules. For the actual "phenotypic" change we still may need an external "stimulator" or may have to wait several cell generations for the level of some intermediate to reach the level needed to evoke the actual change in enzyme concentration we measure.

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## GENETIC RECOMBINATION IN BACTERIA

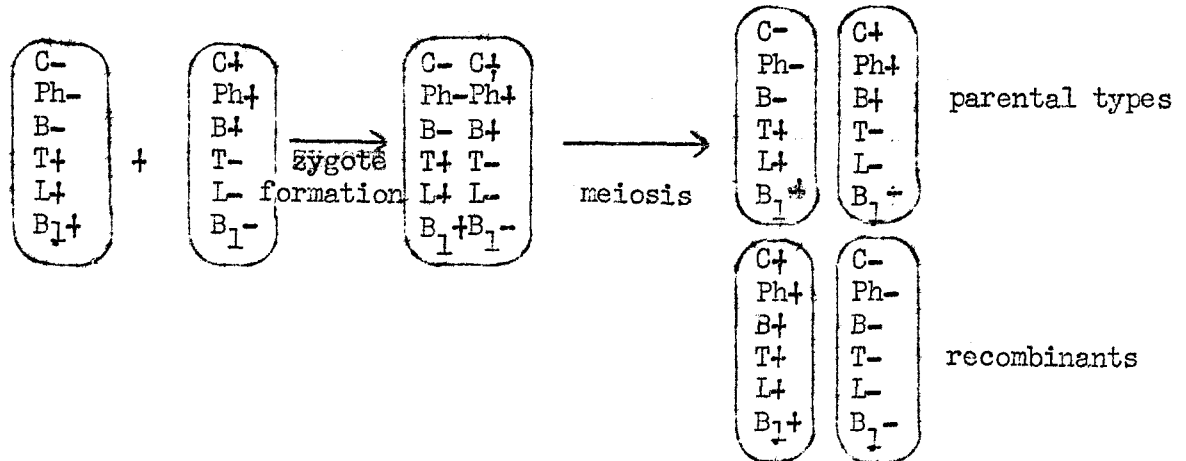
Abstracted by Dale Kaiser and J. J. Weigle

Recombination of hereditary characters in bacteria was first demonstrated in 1946 by J. Lederberg and E.L. Tatum, using strains of *Bacterium coli* K12 which in the wild type can grow on synthetic medium. Their basic experiment was to mix cultures of two strains which had lost this ability to grow on unsupplemented media. One required cysteine, phenylalanine and biotin for growth (represented  $C^{-}Ph^{-}B^{-}$ ) and the other one required leucine, threonine and thiamin ( $L^{-}T^{-}B_1^{-}$ ). Plating the concentrated mixture of the two strains on unsupplemented medium, Lederberg and Tatum observed the growth of colonies. Since these colonies grew on unsupplemented medium (and kept growing on such a medium after transfers), they were not the original parental types; they must have been "recombinants", i.e, from the  $C^{-}Ph^{-}B^{-}$  strain they had inherited  $T^{+}L^{+}B_1^{+}$  and from  $L^{-}T^{-}B_1^{-}$  they had inherited  $C^{+}Ph^{+}B^{+}$ . There was one recombinant for  $10^6$  parental cells.

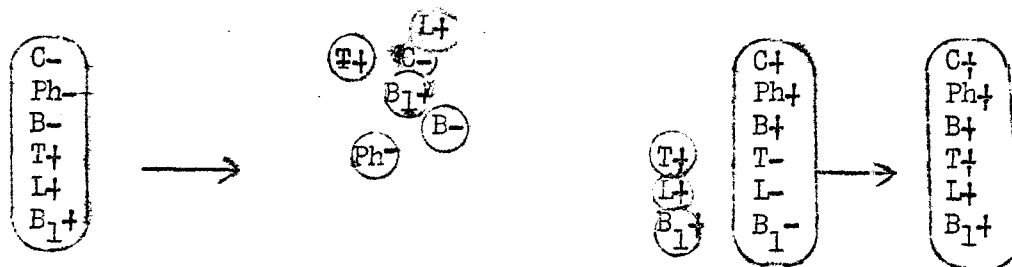
Later the recombination of other characters was studied: fermentation of several sugars, resistance to drugs, resistance to bacteriophages and resistance to colicines. Other strains of coli were also found to undergo recombination. The frequency of recombination in these experiments ranged from 1 in  $10^7$  parental cells to 1 in  $10^4$ .

Two extremely different hypotheses might explain the observed recombination, as follows:

1. Two parental cells fuse to form a zygote containing the genetic material from both parents. Following zygote formation the genetic material is separated again. Before the separation, however, the genetic material of the two parents has had an opportunity to make exchanges. This process of zygote formation followed by meiosis is the one used by higher plants and animals. For the characters used by Lederberg and Tatum the scheme would be as follows.



2. One of the parental types produces agents which upon entrance into the cells of the other parent change its genetic constitution.



Hypothesis (2) differs from (1) in that it is unidirectional, i.e., one of the parents is a donor, the other an acceptor. Thus most of the genetic material of the recombinant will usually have come from the

acceptor parent. This system is analogous to the type transformations in pneumococcus or haemophilus and to transduction in Salmonella.

Since 1946 evidence for the zygote hypothesis (1) has been accumulated by Lederberg, Rothfels, Newcombe and Davis. Recently new facts discovered by Hayes have given strong support to the second hypothesis. What follows is a summary of the evidence.

Facts supporting the zygote hypothesis (1):

A. Cell-free filtrates or extracts of one parent do not have the ability to transform the other parent. (Cell-free filtrates or extracts are known to be active in Salmonella, haemophilus, and pneumococcus.) This fact is not necessarily evidence against hypothesis (2) since it could be argued that the hypothetical cell-free agents might be very labile or might adhere very firmly to the cell.

B. Aberrant recombinants between nutritionally requiring mutants have been found which contain most of the genetic material from both parents. They are stable on unsupplemented medium but on completely supplemented medium they give rise to various lines of descendants. Some of these segregant lines are like the parents, others are recombinants. A simple interpretation of the aberrant recombinants is that they are persistent zygotes--zygotes which failed to undergo meiosis.

C. Simultaneous exchange of several physiologically unrelated characters takes place. In general the tendency of two characters to be inherited together is called linkage. In plants and animals linkage indicates that both characters are on the same chromosome. The following data for E coli K12 exemplifies linkage between M and Lac.

Parents:		Recombinants $M^+L^+T^+$	
$M^-L^+T^+$	$M^+L^-T^-$	$Lac^+$	$Lac^-$
$Lac^+$	$Lac^-$	22.9%	77.1%
$Lac^-$	$Lac^+$	72.4%	27.6%

The first entry says: In the cross  $M^-L^+T^+ Lac^+ \times M^+L^-T^- Lac^-$  22.9% of the recombinants  $M^+L^+T^+$  were  $Lac^+$ , 77.1% were  $Lac^-$ . Thus in selecting for  $M^+$ ,  $Lac^-$  was automatically selected for. The second row shows that this correlation between M and Lac is independent of the state of the Lac character for if the  $M^+$  parent is  $Lac^+$  then the majority of the  $M^+$  recombinants are also  $Lac^+$ . Such simultaneous exchange suggests the transfer of whole units as in crossing over in the chromosomes of other organisms.

Evidence favoring the donor-acceptor hypothesis (2):

A. In the cross  $M^-L^+T^+ \times M^+L^-T^-$ , the fertility (the number of recombinants) is unchanged if the  $M^-$  parent is treated with 1,000 units of streptomycin /ml, in which case the culture of  $M^-$  may become completely sterile (incapable of forming colonies). However the converse is not true: treatment of  $L^-T^-$  with streptomycin reduces fertility parallel with reduction of viable cell count.

B. Small doses of UV light increase the fertility of the  $M^-$  parent but decrease the fertility of the  $L^-T^-$ . A and B indicate that the two parents do not play an equal part in the cross. A and B can be understood if it is assumed that the  $M^-$  parent is the donor and the  $L^-T^-$  parent the acceptor. The  $M^-$  parent, since it has only to yield transforming or transferring agents, would not be sensitive to streptomycin or UV; but the  $L^-T^-$  parent, since it must retain its structure to act as acceptor, would be sensitive to UV and streptomycin.

The increased fertility following UV treatment is observed only if following the treatment the culture is aerated in broth. These conditions are analogous to those required to induce lysogenic bacteria to release their carried phage. This analogy raises two questions:

- a) Is a carried phage responsible for fertility?
- b) Does this phage transfer bacterial genetic material?

The following experimental facts do give at least a partial answer to these questions.

Independently of one another Hayes, Cavalli, and Lederberg found an  $M^-$  strain which would not cross with the  $L^-T^-$  strain, although the original  $M^-$  strain did. This new strain did cross with wild-type and other derived strains. This could be understood if one assumed that there are two classes of parental types called  $F^+$  and  $F^-$  (F standing for "fertility".) Crosses  $F^+ \times F^-$  are fertile,  $F^- \times F^-$  are infertile. Usually  $M^-$  is  $F^+$  and the  $L^-T^-$  is  $F^-$ . The new  $M^-$  variant which does not cross with the  $L^-T^-$  had thus become  $F^-$ .

$M^-F^-$  cells can be transformed to  $M^-F^+$  by short contact with  $F^+$  cells, i.e., cells of strains with which  $M^-F^-$  cells normally cross. If the two strains are grown together in broth with a 25-fold excess of the  $F^+$  strain, 100% of the  $F^-$  cells will be transformed into  $F^+$  cells within 30 minutes. The transformed  $F^+$  cells are stable. Similarly the  $L^-T^-$  can be transformed into an  $F^+$  strain by short contact with  $F^+$  cells. This transformed strain now crosses with the new  $M^-F^-$  variant described above.

On the zygote hypothesis F would be called a "compatibility factor". Such systems are common in fungi and algae. But  $F^+$  could be interpreted

as the carrier of hereditary material on the donor acceptor hypothesis, the donor parent being  $F^+$  and the acceptor  $F^-$ . The  $F^+$  agent resembles phage\* in its infectivity and in its capacity to be propagated in bacterial cells, but differs from phage since it is neither filterable nor pathogenic.

With the new strains crosses can be made with  $F^+$  either in the  $M^-$  or  $L^- T^-$ . The results, which are to be compared with those of Table 1, are:

Parents			Recombinants $M^+ L^+ T^+$	
$M^- L^+ T^+$	x	$M^+ L^- T^-$	<u>Lac<sup>+</sup></u>	<u>Lac<sup>-</sup></u>
$F^+$ Lac <sup>+</sup>		$F^-$ Lac <sup>-</sup>	22.9	77.1
$F^-$ Lac <sup>+</sup>		$F^+$ Lac <sup>-</sup>	85.0	15.0

What was previously interpreted as linkage between M and Lac must now be explained by the "polarity" due to F. This finding gives strong support to the donor-acceptor hypothesis since the frequency of unselected markers (Lac for example) is higher from the  $F^-$  than from the  $F^+$  parent. It shows also that linkage interpretation must be reviewed in terms of the F factor. A greater contribution of genetic material from the  $F^-$  parent is expected on the donor-acceptor hypothesis.

In spite of these facts Lederberg and Cavalli hold to the zygote hypothesis and this for at least two reasons: First, in Salmonella where one way transfer is known the responsible agent is filterable, separable, and characters are independently transferred. In K12 none of these conditions are met. Secondly, a  $M^-$  strain called "High Frequency"

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\* It should be noted that K12 is lysogenic for a phage  $\lambda$  but that the presence or absence of  $\lambda$  does not change the results of the crosses.

exists which yields 100 to 1,000 times more recombinants when crossed with  $L^- T^- F^-$  than the original  $M^- F^+$  strain. However, unlike  $F^+$  strain, Hfr will not transform  $F^-$  to  $F^+$ . However, Hfr is known to revert occasionally to  $F^+$  at room temperature.

Polarity is explained by Lederberg as being due to chromosome elimination during the formation of the  $F^+$  gamete during fertilization, or during meiosis. According to Hayes, polarity results from failure of the F agent to carry all of the genetic material of the donor strain.

In summary it can be said that a carried infective agent is responsible for the fertility of K12 but it is not known whether or not this agent transfers the bacterial genetic material.

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## INDUCED SPECIFIC HERITABLE CHANGES IN BACTERIA

Abstracted by Dorothy K. Fraser and John W. McKee

Zinder and Lederberg have discovered a genetic system in *Salmonella* which is closely related to two types of extracellular hereditary agents--the transforming principles which have been most extensively studied in pneumococcus, and the temperate (lysogenic) bacteriophages which show a remarkable uniformity of behavior, whether investigated in *coli*, *Shigella*, *Megatherium*, *Pseudomonas* or elsewhere.

The *Salmonella* system apparently lies between these two, both of which it closely resembles. A recapitulation of some of the essential properties of each will therefore be given before discussing the *Salmonella* experiments.

### Pneumococcus Transforming Principle

In this system an extract of cells of one strain has the property of changing certain hereditary characteristics of another strain to which it is applied. The agent in the extract responsible for this is desoxyribose nucleic acid. Capsular and somatic antigenic properties and antibiotic resistance are transformable in this way.

#### Rules of the transformation:

- 1) Reciprocal transformation occurs. A strain transformed to carry a new character may later be changed back again with an extract of bacteria possessing the original property.
- 2) The transformed cell gives a transforming principle carrying the new character.

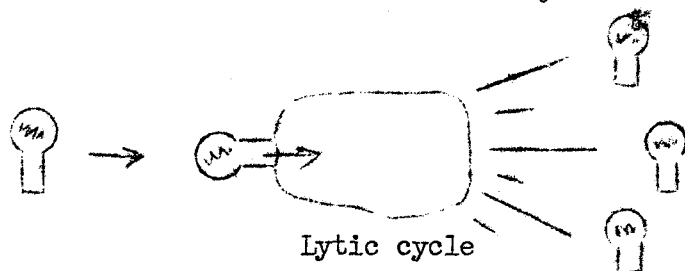


- 3) Exclusion or loss of the original character: The old character is absent both in the phenotype of the cells and in the transforming principle derived from them.
- 4) Individual transformations of single characters are independent, although a single preparation of desoxyribose nucleic acid may be able to transform several characters in appropriate cells.
- 5) Transformation is restricted to characters possessed by donor cells; it is not an unspecific mutagenic effect.

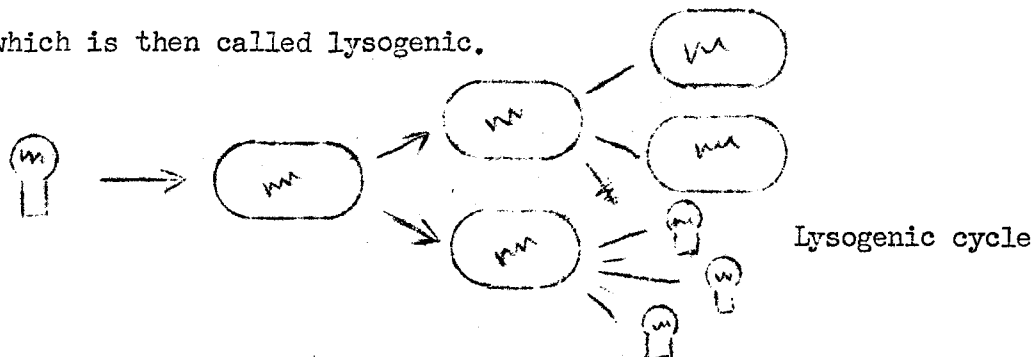
#### Temperate Phage

A bacteriophage is a stable particle which can only multiply within sensitive bacterial cells. It consists of desoxyribose nucleic acid in a protein coat, forming particles of definite size and shape.

Two types of bacteriophage action are to be distinguished. In the lytic cycle the phage is first adsorbed to the cell. The desoxyribose nucleic acid enters the bacterium and sometime later the cell lyses and many new phage come out.



With the temperate phages an alternative action may occur. In this the infecting phage becomes established as a hereditary factor in the cell, which is then called lysogenic.



In its carried state the phage is called a prophage. It is a stable hereditary character of the cell acquired by external infection. From the descendants of an infected cell a greatly increased quantity of infective agent (the phage) can be obtained. The cycle of increase is formally identical with that of the pneumococcus transforming principle. The transformable properties are presence of prophage and related properties such as phage resistance and UV sensitivity, and in E. coli K12 a galactose-fermentation property.

Rules of the transformation:

- 1) No simple process corresponding to reciprocal transformation is known.
- 2) The emergent phage resembles the infecting phage, i.e., it is characteristic of a given prophage.
- 3) Infection of a lysogenic bacterium by a phage closely related to that already carried as a prophage does not necessarily exclude the original. A very complex mess results, but it is clear that both may on occasion be carried together.

"Transduction" in Salmonella

Zinder and Lederberg have worked with strains of Salmonella typhimurium. Most of the experiments have been conducted with an unusually interfertile pair of strains LA2 and LA22. These were derived from strains LT2 and LT22 and are nutritionally deficient as indicated.

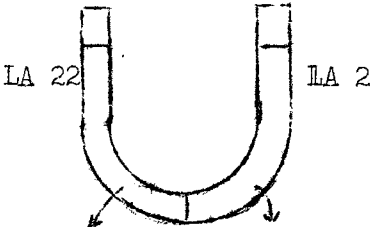
LT2	→	LA2	Methionine, histidine
LT22	→	LA22	Phenylalanine, tyrosine, tryptophan. Unable to ferment galactose, maltose, xylose, mannitol and resistant to streptomycin.

In the basic genetic experiment, suspensions of both strains are grown separately to  $10^9$  cells / ml., washed and plated together on

minimal agar. A few prototrophic colonies grow up. (A prototroph is a bacterium able to form a colony on minimal agar.) Genetic exchange between these two strains therefore occurs.

An experiment first devised by Davis displays very clearly the mechanism of exchange. Cells of the two strains are grown in the two arms of a U-tube separated by a tested bacterial filter. The medium

in the tube is flushed back and forth repeatedly from one side to the other. Finally samples of the contents of both arms are separately plated on minimal medium. Prototrophs are



prototrophs    no prototrophs found in the LA 22 arm of the U-tube only.

It follows that genetic interaction is unidirectional and is transmitted by a filterable agent which Zinder and Lederberg call FA.

A filtrate of LA2 does not produce prototrophs when added to LA22, whereas a filtrate of a mixed culture of LA2 and LA22 does.

Filtered (LA2)	----->	LA22	----->	Prototrophs
Filtered (LA2 + LA22)	----->	LA22	----->	Prototrophs

Therefore LA22 puts out a stimulating agent which causes LA2 to produce FA. LA22 is lysogenic for phage PLT22 and infection with PLT22 is the most effective way to stimulate LA2 to produce FA.

Transferable characters are antibiotic resistance, fermentative ability, loss of growth factor requirements, and presence of somatic and flagellar antigens.

Rules of transduction:

- 1) Possible reciprocal transformations have not been investigated.
- 2) The transduced cell produces a transduced FA.

- 3) There is loss of the original character in both the cell phenotype and the derived FA.
- 4) In any given cross transfer of unselected characters is not observed, even though these are known to be transferable characters. Characters are apparently transferred independently with low efficiency.
- 5) Transfer is limited to the genotype of the cells from which the FA was made. Hence FA is not a nonspecific mutagen.

The very close formal resemblance to pneumococcus transformation is much less apparent when we return to consider the responsible agents.

Stimulating agents: A number of treatments have been tried to obtain FA from LA2, with varying success.

<u>Production of FA</u>	<u>No Production of FA</u>
PLT 22 infection	Complete phage lysis
Dilute antibiotics	Concentrated antibiotics
Crystal violet	Extraction of dried cells
Lithium chloride	Young cultures
Autolysis in aging cultures	Benzene autolysis

The effectiveness of a chemical stimulating agent may be greatly enhanced by the important feature of regeneration. The low yield of FA obtained if reapplied to fresh cells stimulates production of a greatly increased quantity of the agent. By repetition of this regenerative cycle a high concentration of FA may be obtained without the intervention of external Salmonella or phage.



If mutant cells of different genotype are used during regeneration, the FA is characteristic of the cells from which it was immediately derived.

Properties of FA:

- 1) It is released one to three hours after stimulation. The bacteria give typical L-forms.
- 2) It is adsorbed by most smooth strains of Salmonella. Presence of XII<sub>2</sub> somatic antigenic component is required.
- 3) It is affected by various agents:

<u>Inactivate</u>	<u>Do not inactivate</u>
Formalin	Chloroform
70° C. (detectable)	Toluene
100°C. (rapid)	Alcohol
	56° C for 3'
	Pptn by alcohol or $\frac{1}{2}$ sat. $(\text{NH}_4)_2\text{SO}_4$
	Pancreatin
	Trypsin
	Takadiastase
	RNAase
	DNAase

Physically FA resembles much more closely a typical Salmonella phage with its specific protein coat than a DNA transforming principle.

FA is affected by the various agents in the same way as living phage.

- 4) FA is similar to Salmonella phage in size and morphology and in specificity of adsorption. During adsorption and purification the ratio phage/FA remains unchanged.

These facts are the basis for a suggestion by Zinder and Lederberg that a Salmonella phage acts as a passive carrier of FA. As a possible interpretation we might say that these bacteria have formed a trick of

"packaging" their own genetic material for extracellular passage as a transforming principle; the DNA is simply wrapped up in a protein coat, to make which these cells are already completely equipped.



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